APHIS' Evaluation of the Status of High Pathogenicity Avian Influenza H5N1 Virus in Poland

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Abbreviations

ADNS	Animal Disease Notification System
AGID	agar gel immunodiffusion
AI	avian influenza
APHIS	Animal and Plant Health Inspection Service
AU	Administrative Units
BVI	Border Veterinary Inspectorates
CFR	Code of Federal Regulations
CRL	Community Reference Laboratory
CSF	Classical Swine Fever
CVO	Chief Veterinary Officer
DFSVM	Department of Food safety and Veterinary Matters
EC	European Commission
END	exotic Newcastle disease
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FVO	Food and Veterinary Office
GVI	General Veterinary Inspectorate
HI	hemagglutination inhibition
HPAI	highly pathogenic avian influenza
LPAI	low pathogenicity avian influenza
MARD	Ministry of Agriculture and Rural Development
MS	Member State
NVRI	National Veterinary Research Institute
OIE	World Organization for Animal Health
PVI	poviat veterinary inspectorates
PVP	private veterinary practitioners
RT-PCR	real time polymerase chain reaction
RVL	regional veterinary laboratories
SCFCAH	Standing Committee on the Food Chain and Animal Health
SPF	specific pathogen free
TRACES	Trade Control and Expert System
USDA	United States Department of Agriculture
VLA	Veterinary Laboratories Agency
VVI	voivodship veterinary inspectorates

EXECUTIVE SUMMARY

On March 5, 2006, Poland's General Veterinary Inspectorate (GVI) confirmed a case of highly pathogenic avian influenza (HPAI) subtype H5N1 in two dead swans that were found in the city of Torun. The results of the epidemiological investigations suggested that the isolates obtained were closely related to other HPAI H5N1 isolated from wild birds in Europe in 2006. However, no cases of HPAI H5N1 were detected in domestic poultry in 2006 in Poland. The GVI reported the first HPAI H5N1 outbreak in domestic poultry on December 1, 2007. This outbreak was detected in broiler turkeys and between December 1 to December 22, Poland reported a total of ten outbreaks to the World Organization for Animal Health (OIE).

In this document, APHIS presents the results of its evaluation of the HPAI H5N1 status in Poland based on the evaluation of documentation submitted by the GVI, the European Commission (EC), Food and Veterinary Office (FVO) reports, EC legislation, and reports to OIE. APHIS has maintained contact with Polish veterinary authorities who kept APHIS advised of animal disease conditions in their country. In addition, APHIS visited Poland in April 2008 as part of an evaluation of the exotic Newcastle disease (END) in Poland and concludes that a document review is sufficient to meet the needs of this evaluation.

The documentation reviewed was consistent with the OIE Terrestrial Animal Health Code recommendation for reinstatement of trade with a region that has experienced an outbreak of notifiable HPAI. In brief, APHIS based this evaluation on the following critical factors: Poland has been free of HPAI H5N1 for at least 3 months as the result of effective control measures undertaken by the veterinary authorities; that HPAI H5N1 was a notifiable disease in Poland; an ongoing disease awareness program was implemented; all notified or suspect occurrences were investigated; an effective surveillance program for HPAI H5N1 existed that supported the detection and investigation of outbreaks; diagnostic and laboratory capabilities were adequate and effective; eradication and control measures and movement restrictions were appropriate to prevent further spread of disease; and procedures used for repopulation of affected premises included monitoring to demonstrate that HPAI H5N1 had been eradicated.

Wild birds are considered to be the major pathway of introduction of HPAI into Member States of the EU. In the case of an HPAI H5N1 outbreak, eradication activities are needed to mitigate the immediate risk from resulting outbreaks. APHIS considers that the presence of HPAI H5N1 in wild birds presents a high risk for the reintroduction of HPAI H5N1 into Poland. For the purpose of rapid detection of disease, the GVI has in place and maintains an adequate surveillance system for HPAI in wild birds. Extensive surveillance in wild birds and domestic poultry for HPAI H5N1 in Poland since eradication of the outbreaks indicates that it has not been reintroduced. APHIS considers that if HPAI H5N1 were reintroduced into Poland it would be rapidly detected, and appropriate control and eradication measures would be applied to eliminate the disease.

As a result of this evaluation, APHIS concludes that the GVI was able to effectively control and eradicate HPAI H5N1 in its domestic poultry population and that the Polish authorities have adequate control measures in place to rapidly identify, control and eradicate the disease should it be reintroduced into Poland in either wild birds or domestic poultry.

Based on the results of the assessment, APHIS could not identify additional risk factors that would indicate that domestic poultry in Poland continue to be affected with HPAI H5N1. Therefore, APHIS concludes that the likelihood of introducing HPAI H5N1 into the United States through the importation of live birds, poultry carcasses, parts or products of poultry carcasses, and eggs of poultry, game birds or other birds from Poland to be low.

INTRODUCTION

From December 1 to December 22, 2007, the General Veterinary Inspectorate (GVI) reported the detection of ten outbreaks of HPAI H5N1 in two voivodships (provinces) of Poland. The first outbreak was confirmed by Poland's National Reference Laboratory (NRL) in samples taken from turkey flocks for slaughter on December 1, 2007. The outbreak occurred in two holdings in the regions of Myśliborzyce and Uniejewo, district of Plock of the voivodship of Mazowieckie. (See Figure #1 for a map of the outbreaks)

The outbreaks of 2007 in Poland were detected in broiler turkeys, laying hens, backyard flocks and captive birds. Polish authorities took actions to control the spread of disease immediately after confirmation of HPAI H5N1 infection. The GVI concluded that the source of infection was feed contaminated by wild birds in the region. Approximately 700,000 birds were culled during the control measures taken by the GVI. Cleaning and disinfection of affected holdings, equipment and all probable contaminated materials was carried out. Since December 23, 2007, Poland has not reported the presence of HPAI H5N1 in domestic poultry or wild birds.

In response to the outbreaks, and to prevent the introduction of HPAI H5N1 into the United States, APHIS designated the affected regions in Poland as regions where HPAI was considered to exist, and prohibited the importation of birds, poultry, and poultry products from these regions into the United States.

In this document, APHIS presents the results of its evaluation of the HPAI H5N1status of Poland. APHIS is basing this review on the evaluation of documentation submitted by the GVI [1], reports to the European Commission (EC) [2, 3, 4, 5, 6, 7, 15], reports from the EC Food and Veterinary Office (FVO) [8], EC legislation [Appendix 1], and reports to the OIE [9].

On May 1, 2004, Poland and nine other countries became new EU-MS. As part of the accession process, all new MS are required to adopt all EC animal health legislation directly into the MS' animal health regulations, including the regulations pertaining to

AI. Therefore, the EC decisions and directives were transposed directly into Polish law and became the basis for new standard operating procedures by the time of accession. [8]

In 2004, APHIS evaluated the veterinary infrastructure of a number of Member States of the European Union (EU-MS) in an evaluation of the classical swine fever (CSF) status of certain EU-MS. EU-MS were evaluated with regard to the ability to apply the harmonized and binding animal health regulations imposed by the EC. Relevant animal health requirements were evaluated including the compulsory notification of specific animal diseases, including HPAI H5N1, to both the EC and OIE and were found to be adequate. [10]

In 2006, APHIS conducted a risk assessment to evaluate the status of Poland with regard to CSF and swine vesicular disease and concluded that Poland has in place an effective system to identify, control and eradicate animal diseases. Based on this evaluation, APHIS concluded that Poland has implemented EC control measures at a level equivalent to that of the EU-MS evaluated in 2004. [11]

In addition, APHIS identified and presented for public comment what it would consider to be the smallest sub-national jurisdictions or Administrative Units (AUs) in the EU-MS evaluated in 2004. The AU was considered to have "effective oversight of normal animal movements into, out of, and within that jurisdiction, and that, in association with national authorities, if necessary, has effective control over animal movements and animal diseases locally". APHIS recognized that local authorities in these EU-MS have effective oversight and control of animal diseases locally within their respective AUs and in the event of future animal disease outbreaks in the EU, APHIS would regionalize the EU-MS to the level of one or more of the identified AUs [12]. Although the document specifically addressed CSF, the concept of regionalization to the AU level was considered to be more broadly applicable and not disease-specific. In the case of Poland the AU is considered to be the district (*powiats*; singular *powiat*) within the provinces (voivodships). [11]

These evaluations of the veterinary infrastructure, including laboratory capability and its ability to implement appropriate control measures, movement controls, and emergency measures, apply equally to HPAI H5N1. The information provided by Poland regarding HPAI H5N1, in addition to the information from previous evaluations of swine diseases and current evaluations of poultry diseases, provide a background that is consistent with the 11 factor approach in 9 CFR 92.2. APHIS has maintained contact with Polish veterinary authorities who kept APHIS advised of animal disease conditions in their country and concludes that a document review is sufficient to meet the needs of this risk analysis. Also, APHIS visited Poland as part of an evaluation of the exotic Newcastle disease (END) status in April 2008. For these reasons, APHIS concluded that a site visit was not required to complete this evaluation.

Poland provided the information requested by APHIS to support their request for being removed from the APHIS list of H5N1 affected countries. The documentation provided was consistent with recommendations in Article 10.4 of the World Organization for

Animal Health (OIE) Terrestrial Animal Health Code (OIE 2008) for information recommended for reinstatement of trade and HPAI H5N1 free status from a region that has experienced an HPAI H5N1 outbreak. [13] This risk analysis was conducted evaluating information submitted by Polish authorities providing evidence of the following:

- Poland has been HPAI H5N1 free for 3 months because of control measures undertaken by an effective veterinary infrastructure**.**
- HPAI (as defined in 9 CFR) was a notifiable disease in Poland. An ongoing awareness program was in place for veterinary officials and the public, and all notified or suspect occurrences of HPAI H5N1 were subjected to field and laboratory investigations.
- A surveillance program for HPAI H5N1 already existed that addressed Poland's needs. This program supported the detection and investigation of outbreaks, including clinical inspection, active and passive surveillance (both serological and agent detection), and serological and virological testing in high-risk areas and of high-risk flocks. These actions were sufficient to detect disease effectively and quickly, even in the absence of clinical signs.
- Under the surveillance program, all notified and/or suspected avian influenza cases were investigated, and officials took appropriate actions including collecting and transporting these samples in a manner that ensured their integrity for testing purposes, and documenting subsequent laboratory results.
- The system for recording, managing, and analyzing diagnostic and surveillance data was sufficient to demonstrate the effectiveness of Poland's disease control measures.
- Laboratory capabilities were effective, and testing procedures were documented and standardized.
- The eradication program included the definition of appropriate quarantine and surveillance zones, monitoring of those zones, and implementation of movement restrictions. Measures taken by officials were sufficient to contain and control the spread of disease from these zones. Procedures for lifting quarantines were followed and were sufficient to prevent further spread of disease.
- Documented standard operating procedures described procedures for depopulation, cleaning, disinfecting, and other applicable measures, such as carcass disposal. All relevant personnel were familiar with these standard procedures and followed them during the outbreak. These measures were effective in controlling the disease.
- Premises repopulation, if applicable, was carried out according to documented procedures, including evidence that the disease did not recur and monitoring after repopulation to demonstrate that the disease was eradicated.

As a result of this evaluation, APHIS concludes that Poland was able to effectively control and eradicate HPAI H5N1in its domestic poultry population. The effectiveness of the eradication program was attributed to prompt actions taken by GVI. Since the initial outbreak in wild birds that occurred in 2006, Poland has conducted extensive surveillance for HPAI H5N1. Poland has not identified any new HPAI H5N1outbreaks in domestic poultry since December 23, 2007, and on March 31, 2008, declared freedom from HPAI H5N1 to the OIE.

OBJECTIVE

The objective of this report is to evaluate the HPAI H5N1 status of Poland in order to characterize the risk associated with importing live birds, poultry carcasses, parts or products of poultry carcasses, and eggs of poultry, game birds, or other birds from certain regions of Poland, following outbreaks in domestic poultry in 2007.

BACKGROUND

As an EU-MS, Poland is obligated to comply with all EC regulations including those for animal health and disease eradication. Council Directive 2005/94/EC describes the measures for control of AI, and Commission Decision 2004/402/EC requires that all MS develop and implement AI contingency plans to ensure that the most appropriate measures are immediately implemented. Contingency plans and animal disease control measures are harmonized and binding throughout the EU, serving as an important means to prevent the spread of HPAI H5N1 within the EU as well as other countries through its export market. As part of the accession process, Poland presented a contingency plan to the EC which was reviewed and approved by the time of accession. The EC has the authority to conduct periodic evaluations of EU-MS to verify compliance with EC legislation. [8]. The EU system for animal disease control has been extensively evaluated by APHIS for diseases such as CSF and END and provides a basis for understanding the EU system for control of HPAI [10, 11, 12].

HISTORY OF HPAI H5N1 IN POLAND [2-7]

On March 5, 2006, Poland's General Veterinary Inspectorate (GVI) confirmed HPAI H5N1 in two dead swans that were found in the city of Torun. Increased surveillance of wild birds throughout Poland was subsequently undertaken and from March 5 to May 7, 2006, a total of 32 swans tested positive to HPAI H5N1 using real-time polymerase chain reaction (RT-PCR) tests. Surveillance and protection zones were established in several regions of Poland where swans tested positive to HPAI H5N1. In addition, surveillance and protection zones were established in the German Lander (State) of Brandenburg adjacent to an area in Poland where H5N1 had been confirmed in wild birds in the vicinity of the border. On June 7, 2006, the GVI lifted restrictions of the surveillance and protection zones established in several regions of Poland because no other cases of the disease were observed within 30 days from the date of the last confirmed case on May 7, 2006.

On November 30, 2007, Polish authorities sent samples taken from two turkey flocks with clinical signs suggesting HPAI to the National Reference Laboratory (NRL) in Puławy. The turkey flocks originated from Myśliborzyce and Uniejewo; two different regions of the district of Plock, voivoidship of Mazowieckie. On December 1, HPAI H5N1 was identified by RT-PCR, and the GVI applied immediate actions including the establishment of protection and surveillance zones in compliance with Council Directive

2005/94/EC. The same day, the GVI sent official notifications to the EC, the OIE, neighboring countries and trading partners. In Myśliborzyce, a total of 60 turkeys died and 1025 were culled. In Uniejewo a total of 300 turkeys died and 3227 were culled.

On December 3, HPAI was suspected in backyard birds in a neighboring area within the 3 kilometer protection zone established due to the initial outbreak. A total of 15 birds were affected; 2 died and 13 were culled the same day. Samples were taken and the presence of HPAI H5N1 was confirmed the following day.

On December 8, clinical signs were observed leading to suspicion of AI in 6 production units of laying hens in Karniszyn, district of Zuromin, Mazowieckie voivodship. HPAI H5N1 was confirmed the same day and over 100,000 eggs were seized and the production of the recent days was traced. In total, 610 birds died and 118,390 birds were culled on December 9.

On December 10, two captive birds kept in a wild animal shelter in Taftowo, district of Lidzbark, Warminsko-mazurskie voivodship, were found dead and samples were sent to the NRL. The same day, AI was suspected on a commercial holding of laying hens in Sadłowo in the district of Zuromin, Mazowieckie voivodship, and HPAI H5N1 was confirmed. This was considered to be a secondary outbreak and involved a total of 385,407 birds: 209 died and 385,198 were culled.

The NRL confirmed positive results for HPAI H5N1 in the samples taken from the captive birds in Taftowo on December 11 and this was considered a primary outbreak in wild captive birds. A total of 18 birds were involved: 3 birds died and 11 were culled. A total of 4 birds considered to be protected species by the Minister of Environment and were tested by RT-PCR and hemagglutination inhibition (HI) twice with negative results.

On December 12, two outbreaks were confirmed in two backyard holdings in Łępno, not far from Taftowo in the district of Elblag, Warminsko-mazurskie voivodship. A total of 204 birds including hens, ducks and geese were involved in these two outbreaks; 37 died and the remainder were culled the same day.

On December 16, AI was suspected in a backyard holding with 8 birds in Głodówko, a region within the 10 km surveillance zone established for the outbreaks in Łępno. A total of 5 birds died and the NRL confirmed HPAI H5N1 the following day; the remaining 3 birds were culled on December 17.

On December 22, an outbreak was confirmed in production units of laying hens in Sadłowo, district of Zuromin, Mazowieckie voivodship. A total of 20 birds died and 185,415 birds were culled. No other outbreaks were detected after this date.

In summary, a total of 10 outbreaks were detected between December 1 and December 22, 2007 in Poland. A total of two of these outbreaks were in turkey flocks, three in laying hens, four in backyard flocks, and one in wild captive birds. Immediately after confirmation of HPAI, protection zones of 3 km radius and surveillance zones of 10 km

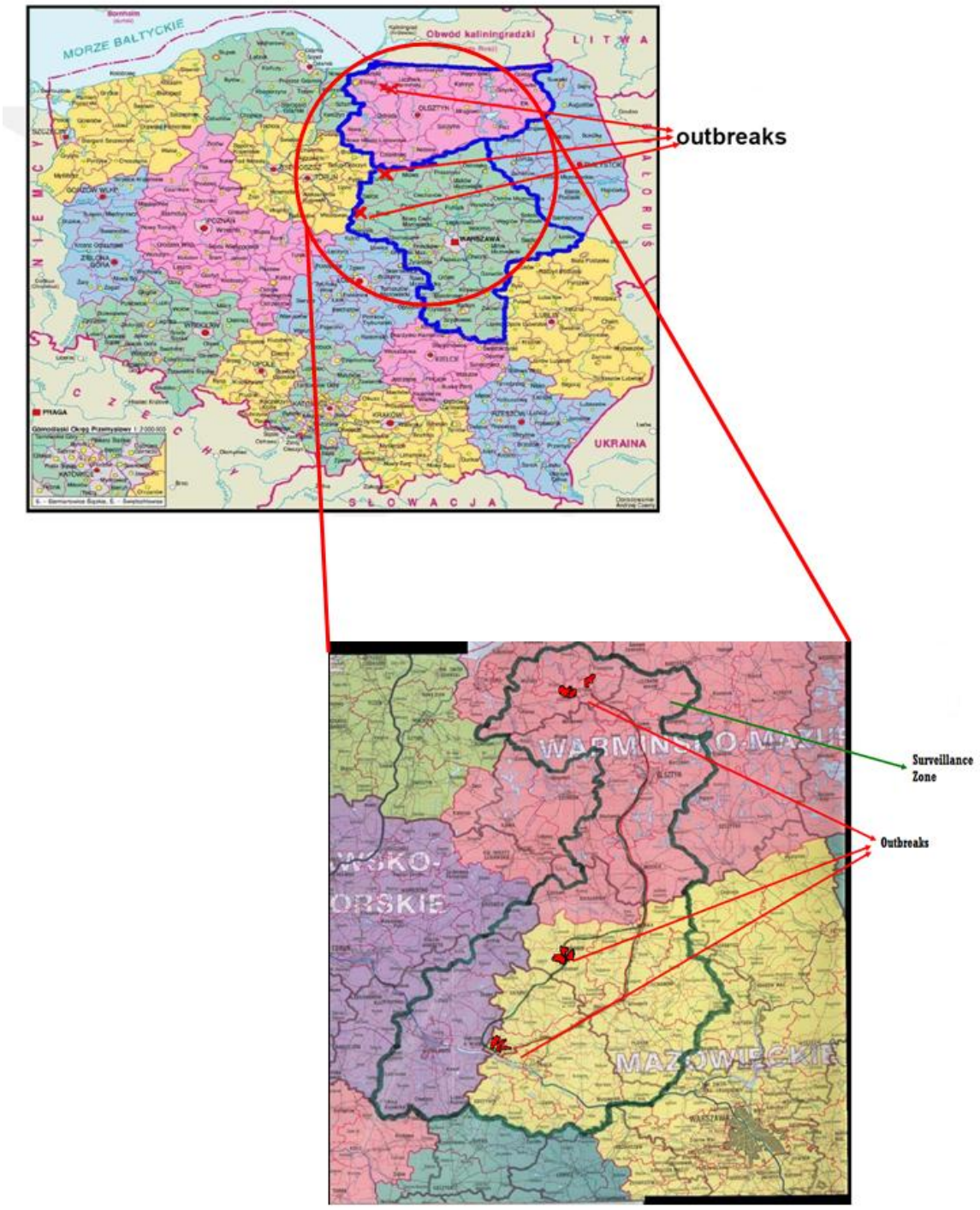
radius were established following control measures described in Commission Decision 2006/415/EC. Control measures were established on a zone along a route used to transport birds to be culled and within the protection and surveillance zones established due to the outbreaks (see Figure 1 for a map of the region of Poland where restrictions were established). In addition, culling, cleaning and disinfection activities were immediately carried out as described by Council Directives 92/40/EC and 2006/563/EC.

On January 21, 2008, Poland lifted restrictions in Warminsko-mazurskie voivodship and some districts in Mazowieckie voivodship. On January 31, Poland lifted the remaining restriction in Mazowieckie voivodship.

Table 1. Summary of the HPAI-H5N1 Outbreaks in Poland.

District/Voivodship	Date	Type	Total No. of birds
MYŚLIBORZYCE /MAZOWIECKIE	12/01/07	turkeys	1085
UNIEJEWO /MAZOWIECKIE	12/01/07	turkeys	3527
MYŚLIBORZYCE /MAZOWIECKIE (secondary outbreak)	12/04/07	backyard hens, ducks	15
KARNISZYN /MAZOWIECKIE	12/08/07	laying hens	119000
SADŁOWO /MAZOWIECKIE (secondary outbreak)	12/10/07	laying hens	385407
KRZYKAŁY /WARMINSKO-MAZURSKIE	12/11/07	captive birds	14
ŁĘPNO /WARMINSKO-MAZURSKIE	12/12/07	backyard hens, ducks	39
ŁĘPNO /WARMINSKO-MAZURSKIE	12/12/07	backyard hens, ducks, geese	165
G ŁODOWKO /WARMINSKO-MAZURSKIE	12/17/07	backyard hens	8
SADŁOWO /MAZOWIECKIE	12/22/07	laying hens	185435

Figure 1. Maps of the regions affected by restrictions due to HPAI outbreaks in Poland in 2007 [4]



HAZARD IDENTIFICATION

APHIS has identified several animal diseases listed by OIE that pose primary hazards associated with initiating trade in animals and animal products from foreign regions. The listed foreign animal diseases of primary concern are addressed specifically in APHIS regulations. One of these diseases, High Pathogenicity Avian Influenza H5N1 (HPAI H5N1) is recognized by APHIS as a hazard of primary concern. In this regard, prior to resumption of trade in poultry and poultry products with a region or country considered by APHIS to have been affected with HPAI H5N1, APHIS must conduct an import risk analysis in support of this action.

Avian influenza (AI) is caused by an orthomyxovirus virus that infects wild birds (such as ducks, gulls, and shorebirds) and domestic poultry (such as chickens, turkeys, ducks, and geese). AI viruses are classified by a combination of two groups of proteins: the hemagglutinin or H proteins, of which there are 16 (H1-H16), and neuraminidase or N proteins, of which there are 9 (N1-N9). AI strains also are divided into two groups based upon the ability of the virus to produce disease (pathogenicity): low pathogenic (LP) and highly pathogenic (HP).

HPAI H5N1, often referred to as "Asian" H5N1", is the type causing worldwide concern. HPAI H5N1 spreads rapidly and is often fatal to chickens and turkeys. Millions of birds have died in countries where HPAI H5N1 has been detected. This virus has also infected people, most of whom have had direct contact with infected birds. "Asian" HPAI H5N1 has not been detected in the United States.

Low pathogenicity (LPAI) H5N1, often referred to as the "North American" H5N1, is of less concern. LPAI H5N1 has been detected in wild birds in the US, as recently as 2007. Other strains, specifically H5N2, of HPAI have been detected and eradicated three times in the United States: in 1924, 1983 and 2004. The 2004 H5N2 isolate did not result in clinical disease and inoculation studies showed it to be a low pathogenic strain; however, genetic sequencing was consistent with one of the OIE definitions for HPAI. No significant human illness resulted from these outbreaks.

ANALYSIS OF THE STATUS OF HPAI H5N1 IN POLAND

Evidence that Poland has been HPAI-free for 3 months because of control measures undertaken by an effective veterinary infrastructure. [1, 8, 14]

Control measures against HPAI and other epizootic diseases are undertaken in Poland as soon as disease is suspected. Preventive and control measures against AI are established at EU level and must be implemented by all MS. Poland became a MS of the EU on May 1, 2004 and since then Poland's competent authorities have implemented prescribed measures if there is a suspect or confirmed case of HPAI in either wild birds or domestic flocks in its territory. A total of ten outbreaks of HPAI H5N1 were detected from December 1 to December 22, 2007. Immediately after confirmation of the disease, the

affected premises were depopulated and a protection area of 3 km radius and surveillance area of 10 km radius were established, pursuant to point 10 of the Commission Decision No 2006/415/EC.

Prior to Poland's accession to the EU, Poland had to fulfill the economic and political conditions of the Copenhagen criteria which basically require that a candidate MS must adhere to a secular, democratic system of government, together with the corresponding freedoms and institutions, and respect the rule of law. In regards to AI, EU-MS may impose more stringent measures if they believe these contribute to a more rapid and effective eradication of the disease, and MSs must keep the Commission informed of such measures.

The rule of law is fundamental to the EU. All EU decisions and procedures are based on the Treaties, which are agreed by all the EU countries. The EC is responsible for ensuring that EU legislation is properly applied, for proposing further legislation to the legislator and for adopting appropriate implementation rules. The EU legislation on animal health issues are adopted by the Council (article 37 of the Treaty). Before these rules can be adopted, these rules are discussed with the MSs experts in the Animal Health and Animal Welfare Section of the Standing Committee on the Food Chain and Animal Health (SCFCAH). The implementation of the measures provided for in the legislation rests with the MSs. The MSs are financially supported by the EU for the expenditure incurred in relation to the measures applied.]

Community measures for the control of AI were established by Council Directive 92/40/EC of May 19, 1992, in order to ensure the protection of animal health and contribute to the development of the poultry sector. A new Council Directive on AI aiming at better prevention and control of outbreaks was adopted by the EU-MS in the Council in December 2005. The new Council Directive 2005/94/EC repeal the Council Directive 92/40/EC and all EU-MS were obliged to implement it by July 1, 2007. The measures laid down in Council Directive 2005/94/EC include comprehensive provisions for the early detection of HPAI infection in poultry to ensure rapid response and adoption of appropriate control and eradication measures.

In addition to the fixed body of legislation laid down for AI, the EC can also adopt additional emergency measures when needed using the AI Directives and other pieces of primary animal health legislation as their legal basis. The Commission, with backing of the EU-MS, can implement legislative measures to address certain issues regarding disease control measures. This is usually done through the SCFCAH, which is made up of members from all EU-MS. Measures taken include import bans, preventive and control measures for specific cases, domestic and wild bird surveillance programs, and defining risk areas around protection and surveillance zones.

Commission Decision 2006/563/EC includes provisions for protection measures in relation to HPAI in wild birds in the Community and sets out the measures to be applied in any EU-MS which has a case of suspected or confirmed HPAI H5N1 virus in wild birds. Protection measures include the establishment of a control area and a surrounding

monitoring area around the positive finding. In addition, on-farm biosecurity measures must be strengthened in the control area, hunting of wild birds is banned, disease awareness of poultry owners must be enhanced, the movement of poultry is banned (except when the movement is directly to the slaughterhouse), and lastly the dispatch of meat outside the zone is forbidden except where products have undergone the controls provided for in EU food controls legislation. These measures are aimed at preventing the spread of HPAI from wild birds to poultry or other captive birds, as well as the contamination of products.

Commission Decision 2005/734/EC include provisions for biosecurity measures to reduce the risk of transmission of HPAI H5N1 from wild birds to poultry and other captive birds, and to provide for a system for early detection in areas at particular risk. This Decision has been amended with additional risk mitigating measures by Commission Decisions 2005/745/EC, 2005/855/EC, 2006/405/EC, 2006/574/EC, amongst others. (See Appendix I for a list of Council Directives and Commission Decisions regarding AI)

Poland has an organizational structure consisting of a central level, 16 Voivodships (province/states), 379 Poviats (districts) and 2,478 Gminas (municipalities). The central level has overall responsibility of animal health activities. Most implementation and enforcement activities are carried out at Voivodship and Poviat levels.

Under the Ministry of Agriculture and Rural Development (MARD), the Department of Food safety and Veterinary Matters (DFSVM) is responsible for presenting legislation to the Parliament of animal health and veterinary issues. The GVI is the competent authority responsible for implementing, enforcing and supervising control measures.

The GVI prepares annual workplans, guidelines and instructions regarding animal health activities for its central, regional and district levels. The GVI is divided into 10 Border Veterinary Inspectorates (BVI), 16 Voivodship Veterinary Inspectorates (VVI) and 304 Poviat Veterinary Inspectorates (PVI).

In April 2007, the GVI reported to the EC a total of 44 veterinarians employed at the central level, 251 at the VVI, 1,476 at the PVI and 68 at the BVI. The GVI also reported a total of 192 veterinarians employed in the official veterinary laboratories. According to Polish authorities, an additional 796 positions were made available for veterinarians in March 2008.

In addition, the PVI designates private veterinary practitioners (PVP) to carry out certain official tasks. In total, there are 5,200 PVP that are certified by the PVI to conduct tasks such as: ante and post mortem examinations at slaughter plants; issuing health certificates; sampling; vaccinations; and other basic tasks in relation to the different disease control programs in Poland.

The National Veterinary Research Institute (NVRI) is the entity responsible for coordinating the activities of the 16 Regional Veterinary laboratories (RVL). Official

laboratory analyses are carried out in accordance with Article 23(3) of the Act on Veterinary Inspection by the 16 RVL, the NRL, and laboratories approved by the Chief Veterinary Officer (CVO). All laboratories are accredited by the Polish Center for Accreditation according to standards described in PN-EN ISO/IEC 17025 (General requirements for the competence of testing and calibration laboratories).

The GVI is the Agency responsible for carrying out veterinary checks in the intra-Community trade of live animals, as well as for approval and supervision of approved sites. The Survey Sampling International (a global company provider of sampling solutions for survey research) has an agreement with the GVI to immediately inform each other about suspect cases of animal disease. This agreement further applies to Voivodships and Poviats.

In 2006, APHIS conducted a risk assessment to evaluate the status of Poland with regard to classical swine fever and swine vesicular disease in which Poland's GVI and its disease identification and control capabilities. APHIS concluded in this risk assessment that Poland has in place an effective system to identify, control and eradicate animal diseases.

A total of 10 outbreaks were detected from December 1 to December 22, 2007 in Poland. Two of these outbreaks were in turkey flocks, three in laying hens, four in backyard flocks, and one in wild birds. Immediate actions were taken upon confirmation of the presence of HPAI to reduce the risk of further transmission. Additional surveillance in the protected areas, depopulation, and cleaning and disinfection of affected holdings were measures taken to eradicate the outbreaks. No cases of HPAI H5N1 have been detected in Poland since December 23, 2007.

Conclusion: APHIS has evaluated the comprehensive set of control measures established by the EC and implemented by the Polish veterinary authorities and concluded that are adequate and effective to control and eradicate HPAI H5N1 in the case of an outbreak. During the HPAI H5N1 outbreaks in 2007, the GVI was able to effectively control and eradicate disease and has not reported HPAI H5N1 since December 23, 2007. APHIS concludes that Poland has been free of HPAI H5N1 in its domestic poultry populations because of control measures undertaken by an effective veterinary infrastructure.

Documentation that HPAI (as defined in 9 CFR) was a notifiable disease in Poland and an ongoing awareness program was in place for veterinary officials and the public, and all notified or suspect occurrences of HPAI were subjected to field and laboratory investigations. [1, 8]

HPAI H5N1 was and is a notifiable disease in Poland under the Act of March 11, 2004, which lays down the regulations in regards to the protection of animal health and combating animal infectious diseases (Journal of Laws No. 69, item 625, as amended). Chapter 8 of this Act describes the legal authorities responsible and the procedures to be taken upon suspicion of infectious diseases including HPAI. In the case of suspicion of

an outbreak of HPAI, the owner of animals is obligated to notify the local office of the Veterinary Inspection, or the relevant administrative authority, or the closest veterinary clinic within 24 hours. An official veterinarian is responsible of taking immediate action to investigate and diagnose the occurrence of the disease.

In addition, as an EU-MS, Poland must notify all suspected or confirmed cases of AI to the EC and to other EU-MS. Council Directive 2005/94/EC requires the competent authorities of all EU-MS to notify each primary outbreak[1] to the Commission within 24 hours, and secondary outbreaks on a weekly basis. Council Directive 92/40/EC establishes that all forms of AI confirmed by the competent authority are considered to be notifiable, and enforces its detection in slaughterhouses, means of transport, border inspection posts and other places at Community borders and quarantine facilities or centers operating in accordance with Community legislation on imports of poultry or other captive birds. The mechanism by which the EU-MS should notify is laid down in Council Directive 82/894/EEC. Detailed information on each outbreak in a MS is sent by the competent authority to the EC via the Animal Disease Notification System (ADNS). There is no differentiation between HPAI and LPAI and both should be notified.

Poland implemented an awareness program entitled "Programme aiming at detection of occurrence of virus infections causing AI and widening knowledge on risk of occurrence of this disease". This program was and is in place for veterinary officials to promote early detection in poultry and wild birds. In April 2008, APHIS visited Poland to evaluate the END status of the country and was able to review first hand the components of the awareness program.

All notified or suspect occurrences of HPAI in Poland are subjected to field and laboratory investigations. Commission Decision 2004/402/EC requires that all EU-MS must initiate an epidemiological investigation to determine: 1) the length of time infection may have existed on the holding; 2) the possible origin of infection on the holding and the identification of other holdings on which there are poultry which may have become infected or contaminated from the same source; and 3) the movement of persons, poultry or other animals, vehicles, eggs, meat and carcasses and any implement or substance likely to have carried AI virus to or from the holding in question. In addition, crisis units are to be established in order to provide full coordination of all field investigations.

Commission Decision 2004/402/EC also requires that all EU-MS have contingency plans for AI in place, and approved by the EC, to ensure that the most appropriate measures are immediately implemented. Poland's contingency plan for AI was approved by the EC and contains a manual of operations and annexes with personnel that must be involved at the regional and district levels in the case of an outbreak. The criteria Poland had to meet to have its AI contingency plan approved by the EC can be found in Appendix 2.

[1] an outbreak that is not epidemiologically linked with a previous outbreak in the same region of a Member State, or the first outbreak in a different region of the same Member State

Conclusion: HPAI H5N1 was and is a notifiable disease in Poland as provided by Act of March 11, 2004 and Council Directive 2005/94/EC. Poland had in place and awareness program for veterinary officials to promote early detection in poultry and wild birds. All notified or suspect occurrences of HPAI were subjected to field and laboratory investigations as required by Commission Decision 2004/402/EC.

A surveillance program for HPAI already existed that addressed Poland's needs. This program supported the detection and investigation of outbreaks, including clinical inspection, active and passive surveillance (both serological and agent detection), and serological and virological testing in high-risk areas and of high-risk flocks. These actions were sufficient to detect disease effectively and quickly, even in the absence of clinical signs. [1, 4, 6, 8, 14]

The EU-MS implement surveillance programs for AI aimed at ensuring early detection of HPAI H5N1. The surveillance and detection systems have been strengthened by investigating increased incidence of morbidity and mortality in wild birds, in particular in selected 'higher risk species'. The criteria used for the identification of 'higher risk species' included factors such as the identification of species that: 1) frequent freshwater wetland habitats and agricultural areas; 2) occur in groups that are large and/or dense; and 3) show a high degree of mixing with other species. In addition the following specific risk factors were considered: likelihood of exhibiting colonial breeding; likelihood of exhibiting predatory behavior; and likelihood of exhibiting scavenging behavior (See Appendix 3.1 for a list of the 'higher risk species').

Anseriformes (water fowl) and *Charadriiformes* (shorebirds and gulls) are the main sampling targets to detect AI. In the event that HPAI H5N1 is detected in wild birds, the EU-MS enhances the surveillance of live and dead wild birds to determine whether these species can act as asymptomatic carriers or 'bridge species'[2] (See Appendix 3.2 for a list of 'bridge species'). These surveillance programs were established under Commission Decisions 2002/649/EC, 2004/111/EC, 2005/464/EC and 2006/101/EC.

Commission Decision 2005/734/EC requires MSs to heighten surveillance and identify poultry holdings located in areas where the risk for disease introduction from wild birds is considered to be higher. Specific risk factors for virus introduction into poultry were identified and include: 1) location of the holding along migratory flight paths of birds from areas where HPAI had been identified; 2) proximity to wet areas, ponds, swamps, lakes or rivers where migratory water fowl may gather; 3) location of the poultry holdings in areas with a high density of migratory birds, particularly waterfowl; and 4) open air holdings of poultry or other captive birds or in any other premises in which

[2] High risk species which may spread H5N1 from wetlands with infected birds to humans and/or poultry, at any time of year. From among the 82 species high risk species, 29 were selected as Bridge Species because they were also considered to pose a relatively high contact risk with humans and/or poultry. This group consists primarily, but not exclusively, of waterbirds, pigeons and doves, corvids and sparrows.

contact between wild birds and poultry or other captive birds cannot be sufficiently prevented.

Decision 2005/731/EC (amended by 2006/52/EC, 2007/105/EC and 2007/803/EC) lays down additional requirements for the surveillance of AI in wild birds to ensure that samples are collected from dead birds and, if possible, from other birds which had contact with dead birds, and that those samples are subject to laboratory tests for detection of the AI virus. If positive laboratory test results for HPAI are obtained, the MS must inform the Commission without delay. All commercial holdings within the protection zone must be visited by an official veterinarian as soon as possible for a clinical examination of the poultry and other captive birds and, if necessary, the collection of samples for laboratory tests in accordance with the diagnostic manual. A record of such visits and the findings thereof must be kept. In addition, non-commercial holdings must be visited by an official veterinarian before the lifting of the protection zone. Additional surveillance is immediately implemented in accordance with the diagnostic manual in order to identify any further spread of AI in the holdings located in the protection zone. Furthermore, records of all persons visiting holdings must be kept by the owner in order to facilitate disease surveillance and control and must be made available upon request by the competent authority.

Poland's surveillance program has been developed following mandates established by. Article 12 of the Ordinance of the Minister of Agriculture and Rural Development of December 17, 2004; the Journal of Laws No. 282, item 2813, which establishes the procedure for conducting controls and the scope of testing of animal infections; and Commission Decision 2005/464/EC of June 21, 2005 concerning the control tests for AI in poultry and game birds in EU-MS.

Poland's surveillance program aims to assess the occurrence of AI infections of H5 and H7 subtype in various poultry species, and to obtain information on risks resulting from the natural environment from which AI viruses may transmit from wild birds to poultry flocks. The regional veterinary officers determine the targeted population to be sampled considering risk factors such as: production types, age, feeding practices, holdings with more than one species per holding, seasonality of the production, seasonality of migratory birds in the region, species behaviors related to migration routes, main habitats, and the results of the previous control programs conducted in the region between 2003-2007.

Passive surveillance of wild birds is conducted in: 1) areas in which increased morbidity or mortality has been observed; 2) areas by the sea, lakes and waters, in which dead birds have been discovered, in particular if these areas are located in a direct vicinity of poultry farms; and 3) bird species posing an high risk to be infected with AI and on the other wild birds species living in their direct vicinity.

Active surveillance is conducted in birds in the absence of clinical signs, clinically ill, injured or hunted birds and it focuses on: 1) migrating birds of *Anseriformes* order (waterfowl) and *Charadriiformes* order (shorebirds and gulls); 2) areas identified as areas of presence of a large number of migratory bird species; 3) areas in which mixing of

various species takes place and in particular on the areas located in a direct vicinity of poultry farms; and 4) other selected species posing an increased risk related to AI.

In the case that H5N1 is detected (by active or passive surveillance) , additional surveillance is conducted aiming to detect symptom-free wild birds carriers by sampling other birds in areas in which epidemiological relationships with the detected cases are present, and other birds that could come into direct contact with poultry farms in high or low risk areas. The results of these tests must be submitted by the NRL to the Chief Veterinary Officer (CVO), the EC and the Community Reference Laboratory (CRL) for AI.

Polish authorities determined that sampling for serological tests will be carried out in three higher risk regions based on the large concentration of poultry and breeding turkeys. These regions are Podlaskie, Świetokryskie, and I warmińsko-mazurskie. Sampling for virological testing in wild birds is carried out mostly in designated regions such as Lubuskie, Podlaskie, Pomorskie, Świetokrzyskie, Warmińsko-mazurskie, Zachodniopomorskie, Dolnoślaskie, Lubeskie, Kujaswsko-pomorskie, and Wielkopolskie.

Active and passive surveillance are conducted using both serology and agent detection. The agar gel immunodiffusion (AGID) and hemagglutination inhibition (HI) tests are commonly and routinely used to detect specific antibodies in poultry and turkey flocks. Virus isolation is carried out using specific pathogen free (SPF) eggs. The procedures of these tests are described later in this document under a section describing the laboratory confirmation capabilities of Poland.

The annual report on surveillance for AI in poultry in the EU reported that in 2007, Poland collected and tested samples from:
• 107 chicken breeder holdings
• 151 laying hen holdings
• 169 turkey fattener holdings
• 17 fattening duck holdings
• 11 breeder duck holdings
• 80 fattening geese holdings
• 49 breeder geese holdings
• 18 game bird holdings
• 28 ratite holdings

Conclusion: Since 2004, Poland has implemented a surveillance program designed to ensure early detection of HPAI H5N1. This program, which includes clinical inspection, active and passive surveillance (both serological and agent detection), and serological and virological testing in high-risk areas and of high-risk flocks, enabled the early detection and subsequent investigation of the outbreaks that occurred in 2007. APHIS considers that Poland's surveillance program was adequate to detect disease effectively and quickly, even in the absence of clinical signs.

Under the surveillance program, all notified and/or suspected AI cases were investigated, and officials took appropriate actions including collecting samples, transporting these samples in a manner that ensured their integrity for testing purposes, and documenting subsequent laboratory results. [1, 4, 6, 7, 8, 14, 15]

Council Directive 2005/94/EC requires that all suspected AI cases must be followed up with an immediate official investigation so that actions including collecting and transporting samples can be taken to confirm or exclude the presence of AI. The investigation includes visits by the official veterinarian to all commercial holdings in the protection zone to conduct a clinical examination of poultry and other captive birds. Non-commercial holdings in the protection zone are also visited by an official veterinarian before lifting of the protection zone. Additional surveillance is immediately implemented to identify any further spread of avian influenza in the holdings located in the protection zone.

In the case of a suspected outbreak, a standard set of samples for virological testing must be collected from at least five sick/dead birds per holding if present, and/or at least 20 tracheal/oropharyngeal and 20 cloacal swabs per holding. In addition, carcasses of birds that have died recently or that are severely sick must be taken. Overall, birds showing clinical signs of disease must be targeted for sampling.

The standard set of samples collected for serological testing must be a minimum of 20 blood samples. Samples must be taken from targeted birds or from all birds on the holding where a smaller number of birds are present. Birds appearing sick or that have apparently recovered must be targeted for sampling. Cloacal and oropharyngeal swabs and/or tissues (e.g., brain, heart, lung, trachea, kidney and intestines) from wild birds found dead or shot are sampled for virus isolation and PCR.

Compulsory surveillance programs are implemented as established by Commission Decision 2007/268/EC to detect the possible circulation of HPAI viruses in poultry flocks and wild birds. It also establishes procedures for storage and transport of samples. These procedures are as follows:
1. Swabs must be chilled immediately on ice or with frozen gel packs and submitted to the laboratory as quickly as possible;
2. Samples must not be frozen unless absolutely necessary;
3. If available, swabs must be placed in antibiotic or specific virus transport medium so that they are fully immersed;
4. Placing samples in medium for transportation must be done in addition to chilling and not as an alternative to chilling;
5. In the absence of such medium, swabs must be returned to their casing and submitted dry;
6. If rapid transport to the laboratory within 48 hours (in transport medium at 4° Celsius) is not guaranteed, samples shall be immediately frozen, stored and then transported on dry ice (See Appendix 4 for the minimum requirements for the transport of samples).

The EC requires under Council Directive 2005/94/EC that EU-MS must ensure that epidemiological investigations are documented with the details of subsequent laboratory

results. In addition, the epidemiological investigation must include details of the length of time during which AI may have been present on the holding or other premises or means of transport; the possible origin of AI; the identification of any contact holding; and the movements of poultry, other captive birds, persons, mammals, vehicles or any material or other means by which the AI virus could have spread.

Poland suspected HPAI on November 30, 2007, due to clinical signs observed in turkey flocks for slaughter in the Mazowieckie voivodship. Positive test results were obtained the following day using RT-PCR in the NRL in Pulawy. Actions to control the spread of disease were taken in compliance with Directive 2005/94/EC immediately after detection, including the implementation of enhanced surveillance in protection zones to detect possible spread of HPAI. The results of these investigations were documented and presented to the EC via weekly reports. The systems for documenting and communicating surveillance data are explained in more detail in the following section of this document.

Conclusion: During the outbreaks in 2007, Polish officials investigated all notified and/or suspected HPAI cases and documented the subsequent results of the investigations. APHIS considers that Poland's procedures for collecting and transporting samples are adequate.

The system for recording, managing, and analyzing diagnostic and surveillance data was sufficient to demonstrate the effectiveness of Poland's disease control measures. [1, 7, 14, 15]

The EC requires that all EU-MS ensure that epidemiological investigations are documented with the details of subsequent laboratory results. The procedures for the confirmation and differential diagnostic of AI, which are carried out in accordance with Commission Decision 2006/437/EC, must be recorded in order to facilitate the management, analysis and communication of surveillance data. The data generated are recorded using several mechanisms such as the Animal Disease Notification System (ADNS) and the Trade Control and Experts System (TRACES).

The ADNS was established by Council Directive 82/894/EC (as last amended by Commission Decision 2004/216/EC) to ensure the notification of animal diseases within the Community. Animal disease notifications are sent to ADNS in accordance with procedures established by Commission Decision 2005/176/EC. All EU-MS must report any primary outbreak to the ADNS within 24 hours of its detection. EU-MS are also required to report secondary outbreaks at least on the first working day of each week.

The EU-MS notifications can be inserted directly into the ADNS via the internet or sent by e-mail to the Commission. This information is automatically recorded into the ADNS system and an e-mail is sent to all the countries connected to the application. The information on primary outbreaks is automatically forwarded by the system to the Commission and to all participating countries, while the information on secondary outbreaks is forwarded once a week by Directorate General of Health & Consumer Protection to all participating countries.

TRACES is a centralized database for the control and traceability of animals and animal products moving within the EU-MS and provides support for disease control measures. TRACES provides electronic certification, data retrieval and central statistical information for imports of animals and animal products. It also allows for the issuance of central risk assessments and warnings for which provides a platform for updated information on disease alerts to be instantly available.

The exchange of surveillance data is also supported by the use of ANIMO, a computerized system which links the veterinary authorities of the EU-MS.

In 2007, a total of 41,631 notifications for all reportable diseases were made by all EU-MS using the ADNS. From this total, 258 notifications were made for HPAI (25 in poultry and 233 in wild birds).

Conclusion: Poland, as an EU-MS, has in place a system to record, manage, and analyze diagnostic and surveillance data that allows for immediate access to information in regard to outbreaks. APHIS concludes that the system in place in Poland is sufficient to demonstrate the effectiveness of Poland's HPAI H5NI control measures.

Laboratory confirmation capabilities were effective, and testing procedures were documented and standardized. [1, 8, 15]

Council Directive 2005/94/EC on Community measures for the control of AI establishes the requirements for the diagnostic procedures, diagnostic manual and reference laboratories in the EU-MS. Testing of samples is carried out at national laboratories for AI or by other laboratories authorized by the competent authorities and under the control of the national laboratories. The national reference laboratory (NRL) in Poland is the Poultry Diseases Laboratory of the National Veterinary Research Institute located in Puławy. All results (both serological and virological) are sent for collation to the AI Community Reference Laboratory (CRL). The CRL for AI is the Veterinary Laboratories Agency (VLA) in Surey, United Kingdom, and provides technical support and maintains a stock of diagnostic reagents. All AI virus isolates are submitted to the VLA in accordance with Community legislation.

The VLA, in consultation with the Commission, coordinates the methods employed by MSs for diagnosing AI. The VLA also types, stores, and supplies strains of AI virus for serological tests and the preparation of antisera. In addition, it supplies standard sera and other reference reagents to the national reference laboratories in order to standardize the tests and reagents used in all EU-MS. Furthermore, it collects and collates data and information on the methods of diagnosis used and the results of tests carried out in the Community. The VLA is also responsible of ensuring that the laboratory testing to detect the presence of AI, and the identification of the genetic type of virus isolates are carried out in each MS according with the diagnostic manual.

Poland's NRL uses RT-PCR diagnostic tests for screening purposes and virus isolation for confirmation. The RT-PCR diagnostic methods were coordinated by the VLA in conformity to the methods required by Council Directive 2005/94/EC. Samples may also be screened using HI tests in accordance with Council Directive 92/40/EC, using antigens and control sera provided by the VLA. (See Appendix 5 for information related to Poland's NRL testing procedures.)

On December 1, 2007, Poland's NRL obtained positive results of HPAI H5N1 in samples screened by RT-PCR. In response to the outbreaks, the GVI applied the protection measures set out in the AI Directive and in Commission Decision 2006/415/EC for domestic poultry. Poland's NRL conducted diagnostic tests of samples taken as a result of an enhanced surveillance in the protection zones. The testing procedures and results of these tests were standardized and documented according to EC legislation.

Conclusion: APHIS considers that Poland's National Reference Laboratory has in place effective testing procedures and was capable of quickly detecting and confirming HPAI H5N1 during the outbreaks in 2007. During the period between December 1 to the last confirmed outbreak on December 22, all tests conducted were documented and its procedures were standard following requirements of Council Directive 2005/94/EC.

Emergency control, biosecurity procedures and eradication program: The eradication program included the definition of appropriate quarantine and surveillance zones, monitoring of those zones, and implementation of movement restrictions. Measures taken by officials were able to contain and control the spread of disease from these zones due to effective program measures. Procedures for lifting quarantines were followed and were sufficient to prevent further spread of disease. [1, 4, 6, 7, 8, 14, 15]

Poland's AI contingency plan fulfilled the criteria established in Directives 92/40/EEC and 92/66/EEC, and was approved in Commission Decision 2004/402/EC. Contingency plans of EU-MS are required to be periodically reviewed and updated if needed under Council Directive 2005/94/EC. Directives 92/40/EEC and 92/66/EEC establishes the criteria needed to be included in contingency plans (See Appendix 2 for the criteria required by the EC for contingency plans).

All EU-MS must take emergency control measures in the case of a suspected outbreak. For example, Commission Decision 2006/416/EC require that when AI is suspected, the competent authority of the MS must immediately ensure that all poultry and other captive birds are brought inside a building on their holding and kept there. Where this is impractical, or if their welfare is compromised, poultry or captive birds must be confined in some other place on the same holding such that they do not have contact with other poultry or other captive birds on other holdings. These measures further minimize their contact with wild birds. In addition, no poultry or other captive birds may enter or leave the holding. Also, no carcasses of poultry or other captive birds, eggs, meat of poultry including offal, poultry feed, utensils, materials, waste, droppings, poultry or other captive birds manure, used litter or anything likely to transmit AI may leave the holding

without an authorization from the competent authority. Furthermore, the movement of persons, mammals of domestic species, vehicles and equipment to or from the holding is subject to the conditions and authorization of the competent authority.

Commission Decision 2006/416/EC requires that a list must be created with the approximate numbers of poultry, captive birds, and mammals of domestic species that are already sick, dead or likely to be infected in each category on the holding immediately upon suspicion of AI. This list must be updated daily to take account of hatchings, births and deaths throughout the period of the suspected outbreak.

Immediately following the confirmation of HPAI, the competent authority of a MS must establish a protection zone with a radius of at least 3 kilometers, and a surveillance zone with a radius of at least 10 kilometers around the holding, as directed by Commission Decision No 2006/415/EC. The protection and surveillance zones are established taking into account several factors such as the geographical situation, natural boundaries, the location and proximity of holdings and the estimated number of poultry, patterns of movements and trade in poultry, the presence of other captive birds in the area, and the facilities and personnel available to control any movement within the protection and surveillance zones. The competent authority may establish additional restricted zones around or adjacent to the protection and surveillance zones based on the criteria above. Restriction zones may extend into neighboring EU-MS when appropriate. The competent authorities of all EU-MS that share a control zone due to an outbreak collaborate to establish these zones.

Commission Decision 2006/416/EC lays down a set of control measures that MSs must take in the protection zone after confirmation of HPAI in poultry. These control measures include: 1) all commercial holdings must be visited by an official veterinarian as soon as possible for a clinical examination of the poultry and other captive birds and, if necessary, samples for laboratory tests must be collected in accordance with the diagnostic manual; 2) a record of such visits and the findings must be kept; 3) non-commercial holdings must be visited by an official veterinarian before the lifting of restrictions of the protection zone; and 4) additional surveillance must be immediately implemented in accordance with the diagnostic manual in order to identify any further spread of AI in the holdings located in the protection zone.

Furthermore, Commission Decision 2006/416/EC prohibits the removal or spreading of used litter, manure or slurry from holdings, fairs, markets, shows or other gatherings of poultry or other captive birds in protection zones. Movements of live poultry, live birds, hatching eggs, meat and meat products of wild feathered game and animal by-products of avian origin must be halted and certain movements may be authorized under stringent veterinary control. Strict biosecurity are applied and means of transport are cleaned and disinfected before and after use.

Commission Decision 2006/416/EC also requires that all carcasses and eggs on the holding in which AI is confirmed shall be disposed of under official supervision. Poultry already hatched from eggs collected from the holding during the period between the

probable date of introduction of HPAI on the holding and the application of the measures must be placed under official supervision and investigations shall be carried out in accordance with the diagnostic manual.

Following the confirmation of HPAI, EU-MS are also required to apply appropriate means of disinfection at the entrances and exits of buildings housing poultry or other captive birds and of the holding itself. In addition, measures must be applied within the protection and surveillance zones including: 1) the establishment of a system to trace all susceptible animals or materials that could have been in contact with infected poultry, captive birds or vehicles linked to the poultry industry; 2) all owners are required to provide the competent authority with records concerning the poultry or other captive birds and eggs entering or leaving the holding; and 3) the implementation of communication through warning notices and the use of the media such as the press and television or any other appropriate means to ensure that all persons in the protection and surveillance zones affected by the restrictions are fully aware of the restrictions in place.

EU-MS can impose more stringent measures if they believe these contribute to a more rapid and effective eradication of the disease, and must keep the Commission informed of such measures.

In Poland, the District Veterinary Officer is the authority responsible of outbreaks confined to the district level; however, the Regional Veterinary Officer takes control when the outbreak spreads to another district, and the central office takes overall control when it spreads between regions.

During the HPAI outbreaks in Poland in 2007, the GVI implemented an eradication program in compliance with Council Directive 2005/94 which included preventive culling of poultry or other captive birds in holdings and areas at risk. These measures were submitted periodically to the EC, which reviews and communicates the situation to all MS and other countries.

Conclusion: APHIS considers that the system established by the EC to approve contingency plans is adequate and ensures that MSs describe the emergency controls, biosecurity procedures and eradication programs for HPAI to be carried out in the case of an outbreak. During the outbreaks in December 2007, the GVI established appropriate quarantine and surveillance zones and was able to contain and control the spread of HPAI. APHIS also considers that the emergency controls, biosecurity and eradication procedures taken during the outbreaks were adequate to contain and control the spread of disease. In addition, the measures taken by Polish officials before lifting quarantines, including the monitoring of the restricted zones, were sufficient to prevent further spread of disease.

Documented standard operating procedures described procedures for depopulation, cleaning, disinfecting, and other applicable measures, such as carcass disposal. All relevant personnel were familiar with these standard procedures and followed them during the outbreak. These measures were effective in controlling the disease. [1, 4, 14]

Competent authorities of the EU-MS and the personnel involved in depopulation activities are required to establish an action plan to ensure compliance with EC regulations established for depopulation procedures during HPAI outbreaks. The standard operating procedures for depopulation are included in the contingency plans that EU-MS are required to have under Community legislation. The operational activities are led by an official veterinarian who has the authority to appoint the personnel to conduct these activities and ensure that they adhere to the required animal welfare and biosecurity standards.

When an AI outbreak is confirmed, the competent authority of an EU-MS must ensure that all poultry and other captive birds on the affected holding are killed without delay and under official supervision as directed by Council Directive 2005/94/EC.

EU-MS may obtain derogations for certain species of poultry or other captive birds not to be killed. For example, Poland detected HPAI H5N1 in a wild animal shelter in December 11, 2007, and a derogation to not kill 4 birds was granted. The derogation was for birds of species protected by the Minister of Environment. These birds were tested twice using RT-PCR and HI with negative results both times.

Council Directive 2005/94/EC requires that all EU-MS must ensure that the vehicles and equipment used for the transport of poultry or carcasses are cleaned and disinfected without delay following the transport by one or more of the procedures set down in Article 48 of this Directive. The procedures for cleaning and disinfecting an infected holding are as follows:

1. As soon as the carcasses have been removed for disposal, those parts of the premises in which the poultry was housed and any parts of other buildings, yards etc. contaminated during slaughter or post-mortem examination should be sprayed with disinfectants approved for use in accordance with Article 11 of Council Directive 2005/94/EC.
2. Any tissue of poultry or eggs which could have contaminated buildings, yards, utensils, etc. should be carefully collected and disposed of with the carcasses.
3. The used disinfectant must remain on the surface for at least 24 hours.
4. Grease and dirt should be removed from all surfaces by the application of a degreasing agent and washed with water.
5. After washing with water, further spraying with disinfectant should be applied.
6. After seven days the premises should be treated with a degreasing agent, rinsed with cold water, sprayed with disinfectant and rinsed again with water.
7. Used litter and manure must be treated by a method capable of killing the virus. This method must comprise one of the following practices:

- incineration or steam treatment at a temperature of 70 C;
- burying deep enough to prevent access by vermin and wild birds;
- stacking and dampening (if necessary to facilitate fermentation), covering to keep in the heat so that a temperature of 20 C is attained and leaving covered for 42 days so as to prevent access by vermin and wild birds.

Conclusion: APHIS considers that the EC legislation establishes adequate standard procedures for depopulation, cleaning and disinfecting of holdings during an HPAI outbreak. During the outbreaks of 2007 in Poland, the GVI and all relevant personnel were able to apply these procedures and effectively control the spread of HPAI and eradicate the disease.

Premises repopulation, if applicable, was carried out according to documented procedures, including evidence that the disease did not recur and monitoring after repopulation to demonstrate that the disease was eradicated. [1, 4, 14]

Commission Decision 2006/437/EC describes the procedures for repopulation of holdings in which HPAI was previously detected and culling, cleaning and disinfection was carried out. The repopulation of commercial poultry holdings must not take place for a period of 21 days following the date of completion of the final cleansing and disinfection as provided for in Article 48 of Council Directive 2005/94/EC. Once commercial poultry holdings are repopulated, the competent authorities of EU-MS must conduct the following activities during a period of 21 days following the date of the repopulation:

1. A check of the production and health records of the holding.
2. A clinical inspection in each production unit, including an evaluation of its clinical history and clinical examinations of the poultry or other captive birds, in particular those that appear sick.
3. Instead of the standard samples, the following samples must be taken from each production unit:
    a. at least 20 blood samples as soon as the poultry have been placed in the holding except in the case of day-old chicks; if appropriate such sampling may be performed on the holding of origin of the poultry before movement to the holding for re-population;
    b. samples of dead poultry or swabs taken from their carcasses from a maximum of 10 dead birds per week during the 21 day period from the date of the re-population.
4. At least 20 tracheal/oropharyngeal and 20 cloacal swabs must also be taken from waterfowl (ducks/geese) in areas surrounding each production unit, if appropriate, within the last week of the 21 day period from the date of re-population.

Conclusion: APHIS considers that the EC legislation establishes adequate procedures for repopulation of holdings in which HPAI was detected.

RISK FACTORS APPLICABLE TO POLAND

The preceding assessment identified pathways by which HPAI H5N1 was introduced into domestic poultry in Poland from wild birds, and the actions taken by the Polish authorities to control and eradicate the disease from its domestic poultry populations. The response to the HPAI H5N1 outbreaks in Poland in 2007 demonstrates that the Polish authorities have adequate measures in place to rapidly identify, control and eradicate the disease should it be reintroduced into Poland in either wild birds or domestic poultry. The reintroduction of disease into domestic poultry remains a concern when HPAI H5N1 is present in wild or migratory bird populations. However, introduction of HPAI H5N1 into Poland by the identified pathways would only increase the likelihood of exporting infected/contaminated products to the United States if domestic poultry became infected and this infection was not detected prior to export. Furthermore, Poland and the EU have in place ongoing surveillance programs aiming to detect the presence of the disease in wild birds.

APHIS cites the following factors as relevant to the situation in Poland:

- Poland was able detect HPAI effectively and quickly as a result of a surveillance program. HPAI is a notifiable disease in Poland and all notified occurrences are subjected to field and laboratory investigations.
- Wild birds are recognized as the major pathway of introduction of HPAI in EU-MS; However, Poland and all members of the EU have in place an adequate surveillance system for the detection of the presence of HPAI in wild birds.
- Poland was able to effectively control and eradicate the HPAI H5N1 outbreaks in domestic poultry as a result of an effective veterinary infrastructure, prompt actions taken by the GVI, and an effective eradication program.
- Poland has in place standard operating procedures for depopulation, cleaning, disinfecting, and other applicable measures, such as carcass disposal in the case of an outbreak, and these measures were effective in controlling the disease during the outbreaks in 2007.
- On January 21, 2008, Poland lifted restrictions in the Warminsko-mazurskie voivodship and some districts of the Mazowieckie voivodship. On January 31, Poland lifted the remaining restriction in the districts of the Mazowieckie voivodship. Since December 22, 2007, no further detection of HPAI H5N1 in domestic poultry or wild birds has been reported in Poland.

RISK ESTIMATION AND CONCLUSION

Poland was able to rapidly identify, control and successfully eradicate HPAI H5N1 following the 2007 outbreaks. Poland has in place adequate surveillance measures to indicate that the disease has not reoccurred in either wild or domestic bird population. In addition, APHIS considers that if there is a reintroduction, Poland would be able to rapidly identify, control and eradicate the disease.

With the successful eradication of HPAI H5N1 following the outbreaks in Poland in 2007, and the subsequent measures implemented in response to those outbreaks, APHIS could identify no additional risk factors that would justify not removing Poland from the APHIS' list of Countries/Regions Affected with Highly Pathogenic Avian Influenza subtype H5N1. The voivodships (provinces) of Warminsko-Mazurskie, Mazowiekie, and Kujawsko-Pomorskie are the regions in Poland that are currently listed in APHIS' list of Countries/Regions Affected with Highly Pathogenic Avian Influenza subtype H5N1.

APHIS concludes that there is a potential for reintroduction of HPAI H5N1 into Poland's poultry population when HPAI H5N1 is present in the wild or migratory bird populations. However, in consideration of the quick and decisive actions undertaken by Polish authorities following the identification of HPAI H5N1 in wild birds in Poland and its neighboring countries, the measures implemented in Poland, APHIS concludes that, if reintroduced, spread of HPAI in Poland would be limited.

Based on the results from this evaluation, APHIS considers the risk of introducing HPAI H5N1 into the United States from the import of live birds, poultry carcasses, parts or products of poultry carcasses, and eggs of poultry, game birds or other birds from Poland to be low.

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Appendix 1: List of Council Directives and Commission Decisions in force relating to AI

Council Directive 2005/94/EC of 20 December 2005 on Community measures for the control of AI and repealing Directive 92/40/EEC [Official Journal L 10 of 14.1.2006].

Commission Decision of 4 August 2006 approving a Diagnostic Manual for AI as provided for in Council Directive 2005/94/EC [Official Journal L 237 of 31.08.2006].

Specific surveillance and security measures

Commission Decision 2006/563/EC of 11 August 2006 concerning certain protection measures in relation to highly pathogenic AI of subtype H5N1 in wild birds in the Community and repealing Decision 2006/115/EC [Official Journal L 222 of 15.08.2006].

Commission Decision 2006/474/EC of 6 July 2006 concerning measures to prevent the spread of highly pathogenic AI caused by influenza A virus of subtype H5N1 to birds kept in zoos and approved bodies, institutes and centers in the EU-MS and repealing Decision 2005/744/EC [Official Journal L 187 of 08.07.2006].

Commission Decision 2006/416/EC of 14 June 2006 concerning certain transitional measures in relation to highly pathogenic AI in poultry or other captive birds in the Community [Official Journal L 164 of 16.06.2006].

Commission Decision 2006/415/EC concerning certain protection measures in relation to highly pathogenic AI of the subtype H5N1 in poultry in the Community and repealing Decision 2006/135/EC [Official Journal L 164 of 16.06.2006].

Commission Decision 2005/734/EC of 19 October 2005 laying down biosecurity measures to reduce the risk of transmission of highly pathogenic AI caused by Influenza virus A subtype H5N1 from birds living in the wild to poultry and other captive birds and providing for an early detection system in areas at particular risk [Official Journal L 274 of 20.10.2005].
This Decision has been amended by:
Decision 2005/745/EC [Official Journal L 279 of 22.10.2005];
Decision 2005/855/EC [Official Journal L 316 of 02.12.2005];
Decision 2006/405/EC [Official Journal L 158 of 10.06.2006];
Decision 2006/574/EC [Official Journal L 228 of 22.08.2006].

Commission Decision 2005/731/EC of 17 October 2005 laying down additional requirements for the surveillance of AI in wild birds [Official Journal L 274 of 20.10.2005]
This decision was extended by Decision 2006/52/EC [Official Journal L 27 of 01.02.2006]

Implementation of surveys on AI

2005/2006:
Commission Decision 2005/464/EC of 21 June 2005 on the implementation of survey programmes for AI in poultry and wild birds to be carried out in the EU-MS [Official Journal L 164 of 24.6.2005]

2004:
Commission Decision 2004/111/EC of 29 January 2004 on the implementation of surveys for AI in poultry and wild birds in EU-MS, to be carried out during 2004 [Official Journal L 32 of

5.2.2004]
This Decision has been amended by Decision 2004/615/EC [Official Journal L 278 of 27.8.2004].

2002/2003:
Commission Decision 2002/649/EC of 5 August 2002 on the implementation of surveys for AI in poultry and wild birds in the EU-MS [Official Journal L 213 of 9.8.2002]

Approval of surveys on AI

Commission Decision 2006/314/EC of 16 March 2006 approving the EU-MS' survey programmes for AI in poultry and wild birds during 2006 [Official Journal L 116 of 29.04.2006]

Commission Decision 2005/732/EC of 17 October 2005 approving the programmes for the implementation of EU-MS' surveys for AI in poultry and wild birds during 2005 and laying down reporting and eligibility rules for the Community financial contribution to the implementation costs of those programmes [Official Journal L 274 of 20.10.2005].

Commission Decision of 2004/630/EC of 27 July 2004 approving the programmes for the implementation of EU-MS' surveys for AI in poultry and wild birds during 2004 and laying down reporting and eligibility rules for the financial contribution from the Community to the implementation costs of those programmes [Official Journal L 287, 8.9.2004]
This Decision has been amended by Decision 2004/679/EC [Official Journal L 310 of 7.10.2004].

Commission Decision 2002/673/EC of 22 August 2002 approving the programmes for the implementation of EU-MS' surveys for AI in poultry and wild birds [Official Journal L 228, 24.8.2002]
This Decision has been amended by Decision 2003/21/EC [Official Journal L 8 of 14.1.2003].

Community reference laboratory

Commission Decision 96/99/EC of 12 January 1996 on financial aid from the Community for the operation of the Community Reference Laboratory for AI (Central Veterinary Laboratory, Addlestone, United Kingdom) [Official Journal L 23 of 30.1.1996].
This Decision has been amended by Decision 97/421/EC [Official Journal L 179 of 08.07.1997].

Additional information is available on the website of the European Commission's Directorate-General for Health and Consumer Protection .

Appendix 2: Criteria Required by the EC for EU-MS Contingency Plans

Contingency plans shall meet at least the following criteria:

1. the establishment of a crisis center on a national level, which shall coordinate all control measures in the Member State concerned;
2. a list shall be provided of local disease control centers with adequate facilities to coordinate the disease control measures at a local level;
3. detailed information shall be given about the staff involved in control measures, their skills and their responsibilities;
4. each local disease control center must be able to contact rapidly persons/organizations which are directly or indirectly involved in an outbreak;
5. equipment and materials shall be available to carry out the disease control measures properly;
6. detailed instructions shall be provided on action to be taken on suspicion and confirmation of infection or contamination, including proposed means of disposal of carcases;
7. training programmes shall be established to maintain and develop skills in field and administrative procedures;
8. diagnostic laboratories must have facilities for post-mortem examination, the necessary capacity for serology, histology etc. and must maintain the skills for rapid diagnosis. Arrangements must be made for rapid transportation of samples;
9. details shall be provided of the quantity of avian influenza vaccine estimated to be required in the event of a reinstatement of emergency vaccination;
10. provisions shall be made to ensure the legal powers necessary for the implementation of the contingency plans.

Appendix 3:

1. **List of species considered high risk.**
   (adopted on May 12, 2006 by the Panel on animal health and welfare of the European Food Safety Authority (EFSA) as well as on the basis of works conducted by the ORNIS Committee and contractors of the services procured by the European Commission's Environment Directorate) [1]

Common name	**Scientific name**
Tundra swan	Cygnus columbianus
Whooper swan	Cygnus cygnus
Mute swan	Cygnus olor
Gooses	
Pink-footed goose	Anser brachyrhynchus
Bean goose	Anser fabalis
White-fronted goose (European species)	Anser albifrons albifrons
Lesser white-fronted goose	Anser erythropus
Greylag goose	Anser anser
Barnacle goose	Branta leucopsis
Brent goose	Branta bernicla
Red-breasted goose	Branta ruficollis
Canada goose	Branta canadensis
Ducks	
Wigeon	Anas penelope
Common teal	Anas crecca
Mallard	Anas platyrhynchos
Northern pintail	Anas acuta
Garganey	Anas querquedula
Northern shoveler	Anas clypeata
Marbled duck	Marmaronetta angustirostris
Red-crested pochard	Netta rufina
Common pochard	Aythya ferina
Tufted duck	Aythya fuligula
Waders	

Lapwing	Vanellus vanellus
Golden plover	Pluvialis apricaria
Black-tailed godwit	Limosa limosa
Ruff	Philomachus pugnax
Gulls	
Black-headed gull	Larus ridibundus
Common gull	Larus canus

2. List of species considered "bridge species".

English name-Scientific name

Cattle Egret- *Bubulcus ibis*
Grey Heron- *Ardea cinerea*
White Stork- *Ciconia ciconia*
Mute Swan- *Cygnus olor*
Greater White-fronted Goose- *Anser albifrons albifrons*
Greylag Goose- *Anser anser*
Greater Canada Goose- *Branta canadensis*
Eurasian Wigeon- *Anas penelope*
Common Teal- *Anas crecca*
Mallard- *Anas platyrhynchos*
Common Coot- *Fulica atra*
Northern Lapwing- *Vanellus vanellus*
Black-headed Gull- *Larus ridibundus*
Stock Dove- *Columba oenas*
Common Wood Pigeon- *Columba palumbus*
Eurasian Collared Dove- *Streptopelia decaocto*
Barn Swallow- *Hirundo rustica*
Fieldfare- *Turdus pilaris*
Redwing- *Turdus iliacus*
Black-billed Magpie- *Pica pica*
Eurasian Jackdaw- *Corvus monedula*
Rook- *Corvus frugilegus*
Carrion Crow- *Corvus corone*
Hooded Crow- *Corvus cornix*
Common Starling- *Sturnus vulgaris*
Spotless Starling- *Sturnus unicolor*
House Sparrow- *Passer domesticus*
Spanish Sparrow- *Passer hispaniolensis*
Chaffinch- *Fringilla coelebs*

Appendix 4. Minimum safety requirements for the transport of samples

1. The transport of samples in which pathogens are known to be present or suspected of being present are the subject of strict national and international regulations that must be adhered to at all times. Virus isolates are not classified as diagnostic samples but must be packaged in accordance with international standards.
2. Packing diagnostic specimens for transport-Diagnostic specimens transported under the IATA Regulations are assigned to UN identification number 2814, 2900, or 3373, as relevant. The shipper and not the transport company is responsible for the shipment until the package reaches the consignee.
3. Primary packaging
    a. Primary receptacle(s) must be water tight, for example, screw caps must be sealed with parafilm or adhesive tape or similar protections taken.
    b. Multiple primary receptacles must be wrapped individually to prevent breakage.
    c. When determining the volume of diagnostic specimens being shipped, the viral transport media must be taken into account.
    d. Primary receptacle(s) must not contain more than 500 ml or 500 g.

    The entire contents of the primary receptacle is the diagnostic specimen.
4. Secondary packaging
    a. Enough absorbent material in the secondary container to absorb the entire contents of all primary receptacles in case of leakage or damage must be used.
    b. Secondary packaging must meet the IATA packaging requirements for diagnostic specimens including 1,2 meters (3,9 feet) drop test procedure. Since infectious substance packaging surpasses the requirements for diagnosticspecimen packaging, in the IATA Packing Instruction 602, it may be used.

Appendix 5. Test Procedures for HPAI

Preparation of samples for isolation purposes
Samples should be placed in isotonic buffer solution (PBS) of pH value of 7.0-7.4 and containing antibiotics. For tissues and tracheal swabs the following antibiotics are recommended: penicillin (2000 m.u./ml); streptomycin (2 mg/ml); gentamicin (50 µg/ml) and mycostatin (1000 m.u./ml). For feces, intestines content and cloaca swabs, concentration of antibiotics should be five times higher. After adding antibiotics, pH of the solution should be corrected to the value of 7.0-7.4. Fragmented tissues or feces are used to prepare 10-20% (w/v) suspension in the solution containing antibiotics, using a whipper or mortar. After homogenization the suspension should be left for 1 or 2 hours in room temperature.
If the material is not used for isolation purposes immediately, it may be stored in temperature of 4^0C, however not longer than for 48 hours – for longer storage it should be frozen in temperature of -70^0C.

Isolation of virus on chicken embryos
Liquid from over the suspension (supernatant) obtained from centrifugation (app. 1000 x g for 10 minutes in temperature not exceeding 25^0C) of the suspension of tissues or feces introduced in the amount of 0.2 ml to allantoic sac of 9-11 days old SPF embryos, using at least 4 embryos for each trial. Inoculated embryos are incubated in temperature of 35-37^0C up to 7 days, examining them by fluoroscopy every day. Both dead and living embryos are cooled in temperature of 4^0C to the end of observation period, then the amniotic – allantoic fluid taken from them is tested for hemagglutination activity (see part II.2.b.). In case of a negative result, at least one additional passage is made, using undiluted allantoic fluid for embryos infection. If hemagglutination is stated in infected embryos' fluids, indicating also on the presence of bacteria, such fluid may be used for further passage, after its filtering (450 nm) and adding antibiotics.

Virus identification
Hemagglutination activity - HA (HA+) detected in bacteriologically sterile fluids taken from the infected embryos may be caused by each of 15 subtypes of AI A or 9 serotypes of paramyxoviruses of birds, including Newcastle disease virus (ND). Therefore each isolated virus of hemagglutinative properties is subject to further tests timing AT its identification and determining pathogenicity.
Identification of A influenza virus is of two-phase nature:

1. Detecting of group-specific antigens (NP and/or MP) common for every A influenza viruses in agar gel immunodiffusion test (AGID) using a method described in part II.2.a.). Antigens prepared by means of concentration of virus from amniotic fluid or extraction of amniochorial membranes of the infected embryos is tested in a presence of known positive serum. Also the immunofluorescence method or ELISA may be used to detect group antigens;
2. Determination of affinity of the insulator to A influenza virus subtype on the basis of H surface antigens, is being conducted by using hemagglutination inhibition (HI) in a way described in a part II.2.b.) in a presence of polyclonal specific serums at least for H5 and H7 subtypes of AI virus. The result of identification is considered as positive, if serum specific for a given subtype has a HI titer of at least 2^4 (1/16) with the tested HA+ virus isolate. Due to importance of AI viruses of H5 and H7 subtypes in HPAI pathology, the reference laboratory must for the most part identify these two subtypes of the AI virus.

Differential diagnostics should consider paramyxoviruses of birds, including definitely the ND virus (paramyxovirus of birds' type 1, PVM-1). For this purpose each HA+ insulator is being tested using hemagglutination inhibition test (HI) in a presence of serum monospecific for the ND virus.

Determination of pathogenicity virus
Due to significant differentiation of A influenza viruses pathogenicity isolated from birds, the basis of the HPAI laboratory diagnostics is determination of the pathogenicity of the isolated virus *in vivo*. It was however stated that the viruses proving its low pathogenicity in laboratory tests, became highly pathogenic in a result of a mutation. Potential possibility of increase in (growth) pathogenicity is generally noticeable by H5 and H7 subtypes and this is connected to amine aminoacids sequence in a place of hemagglutinin cut-off.

Pathogenicity tests:

a. Intravenous Pathogenicity Index (IVPI)
Infectious allantoic fluid from the possibly lowest passage (the best from the initial one) is diluted in 1/10 in sterile PBS and introduces intravenously in the amount of 0.1 ml to ten 6-weeks of age SPF chickens. The infected birds are being observed every day for the period of 10 days. The IVPI index is calculated on the basis of the following evaluation grade for each bird: 0-healthy; 1-ill; 2-seriously ill (breathlessness, apathy, diarrhea, and lividity of unfledged skin parts or appendixes, head swelling, nervous symptoms); 3-dead. IVPI index of 3.0 means that all birds were dead within 24 hours after infection, whereas the 0.0 index means that none of birds was ill and dead within the 10-days long observation.

b. Ability to create transluscences
The evaluation is conducted by using fibroblast cells of chicken embryo (CEF) or relevant laboratory line, e.g. Madin – Darby cattle kidney, which are infected by a series of tenfold dilutions of a virus in PBS (up to 10^{-7}). For a single-layer cells culture prepared in Petri dishes of 5 cm diameter, 0.2 ml of each dilution are placed, provided that 2 dishes are taken for dilution purposes, and are adsorbed for 30 minutes. Then, after threefold rinsing of PBS the cultures are covered by agar base composed of nutritive fluid specific for the cells and 1% (w/v) agar containing 0.01 mg / ml of trypsin or without trypsin. Agar base may not contain serum. After incubation in temperature of 37^0C for 72 hours the agar layer is being removed and the cells are colored using 0.5% (w/v) crystal violet in 25% ethanol.

All viruses incubated in agar base containing trypsin create translucencies, whereas in case of lack of trypsin in agar base the translucence are generated only by the viruses pathogenic for chickens.All isolates of A influenza viruses of H5 and H7 subtypes as well as of the other subtypes demonstrating growth in *in vitro* culture without trypsin, should be immediately delivered by the national reference laboratory to the reference laboratory of OIE or EU for complex characteristics, including determination of sequence of nucleotides of hemagglutinin gen.

Serological tests

a. Immunodiffusion in agar gel
The AGID is commonly and routinely used to detect specific antibodies in hens and Turkey flocks as the infection identifier. Antigen containing NP and M group-specific proteins prepared from the chorionic membranes of chicken embryos infected in 10^{th} day of living is the most often used one. The membranes after homogenization are frozen and defrosted three times, and then centrifuged by 1000xg. The fluid collected from over the suspension is inactivated by adding 0.1% formalin or 1% of beta-propiolakton, centrifuged again and used as antigen.

The test is being conducted using 1% (w/v) agarosis or refined agar containing (w/v) NaCl in phosphate buffer of pH of 7.2, which is placed on Petri dishes or basic glass of 2 – 3 mm thickness. In the congealed agar holes of app. 5 mm diameter of a distance of 2 – 5 mm between them are cut. The holes are filled with app. 0.05 ml of each reagent: antigen, known positive serum and tested serums. Precipitation lines are detected after 24 – 48 hours, which is dependent on concentration of both the antibodies and antigen.

The specific reaction is considered as positive, when a precipitation line between the antigen and positive serum, connects in continuous manner with a line between the antigen and the tested serum. Crossing of lines results from lack of identity of the tested serum with the antibodies of the known positive serum.

Hemagglutination and hemagglutination inhibition tests
Both the HA and HI tests are conducted in micro-dishes of V type and the final volumes of the applied reagents amount 0.075 ml. The following reagents are used for tests purposes: inactivated antigen of AI virus of H5 and H7 subtype, isotonic PBS (0.1M) of 7.0 = 7.2 pH and erythrocytes taken from at least 3 SPF chickens (or regularly monitored birds having no antibodies against AI viruses) to the equal volume of Alsever solution; erythrocytes should be rinsed three times in PBS before their using as 1% (V/V) suspension in PBS. In each conducted HI test, both positive and negative control serum is being used.

HA test. To the dilutions increasing twice (from 1/2 to 1/2048) of the antigen (0.025 ml/hole) prepared in PBS 0.025 ml of PBS are added and then 0.025 ml of 1% (v/v) erythrocytes suspension. After gentle mixing the dish is left to the moment of drop ping of the blood cells on the bottom of the hole for app. 40

minutes in room temperature (app. 20 ^{0}C) or app. 60 minutes in temperature of 4^{0}C (in cases of too high temperature). Titer of HA shall be understood as the highest dilution of the antigen providing full hemagglutination (1 HA unit). In order to more detailed determination of the HA titer of an antigen it is recommended to apply threefold repetitions or different output dilutions e.g. 1/3, 1/4 and 1/5.

HI test. To the dilutions of serum increasing twice (0.025 / hole) prepared in PBS, 4 units of HA of the A influenza virus antigen in the volume of 0.025 ml of 1% (v/v) suspension of erythrocytes are addend and left after mixing for at least 30 minutes in room temperature (app. 20^{0}C) or 60 minutes in temperature of 4^{0}C. Then 0.025 ml of 1% (v/v) of erythrocytes suspension are added and left after mixing to the moment of full dropping of blood cells on the bottom of the hole (app. 40 minutes) in room temperature (app. 20^{0}C) or in 4^{0}C, if the surrounding temperature is higher.
Serum is considered positive, if its HI titer is HI 2^4 (1/16) and higher in a presence of 4 units of the HA antigen.

c. ELISA test
An additional test to detect AI-virus specific antibodies is the ELISA test. This one, similarly to the AGID test, detects antibodies against group-specific antigens.
Introduction of the ELISA test to use in the local diagnostic laboratories requires attestation of the national reference laboratory.
Execution of the ELISA test should be compliant with the procedure provided by the set manufacturer.